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Award Number: DAMD17-98-1-8640

TITLE: The Generation and Preclinical Evaluation of Homodimeric
Anti-Her-2 Antibodies

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REPORT DATE: April 2002

TYPE OF REPORT: Final Addendum

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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20030220 092

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE April 2002	3. REPORT TYPE AND DATES COVERED Final Addendum (1 Apr 01 - 31 Mar 02)	
4. TITLE AND SUBTITLE The Generation and Preclinical Evaluation of Homodimeric Anti-Her-2 Antibodies			5. FUNDING NUMBERS DAMD17-98-1-8640	
6. AUTHOR(S) Ellen S. Vitetta, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The University of Texas Southwestern Medical Center at Dallas Dallas, Texas 75235-9105 E-MAIL: Ellen.Vitetta@UTSouthwestern.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) Her-2/neu has been selected as a target for immunotherapy based on its prevalence on a variety of tumor cells and the poor prognosis associated with its overexpression. From a panel of a hundred α -Her-2 MAbs we have characterized eleven with the highest affinity for Her-2 for further study in the treatment of prostate cancer. Three were eventually chosen for testing as homodimers and all induced substantially more growth arrest and death <i>in vitro</i> than their corresponding monomers. Work has also begun converting a number of these MAbs into mouse/human chimeric IgG1/ κ into which we have incorporated cysteine residues for homodimer formation. These genetic dimers will be evaluated for efficacy in our <i>in vitro</i> models.				
14. SUBJECT TERMS prostate cancer, Her-2, preclinical evaluation				15. NUMBER OF PAGES 10
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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INTRODUCTION

Her-2 is the signaling transduction subunit of the Neu differentiation factor receptor (NDFR) which is comprised of Her-2 and either Her-3 or Her-4, two additional members of the EGFR family which are capable of binding NDF but are not capable of signaling (1). In carcinomas of the breast, ovary, colon and prostate, Her-2 and other components of the NDFR signaling cascade can be highly overexpressed, leading to dysregulated cell proliferation (2,3) and increased metastasis (4,5). Indeed, patients with Her-2 overexpressing tumors have the poorest prognosis (4,6,7). Antibody-mediated immunotherapy is emerging as a powerful tool to augment the effects of conventional therapy (8-10). Indeed, the first MAb for cancer therapy was approved by the FDA in July 1997. Until recently, it was assumed that most antibodies worked by virtue of their effector function, *i.e.*, ability to fix complement or recruit cytoplasmic effector cells (11-14). Based on the work which we began several years ago on lymphoma and have since extended to breast and prostate cancer, it is becoming clear that the most effective MAbs usually signal growth arrest or apoptosis in tumor cells (15-17). Importantly, negative signaling is related to hypercrosslinking, since in our studies with human lymphoma and breast carcinoma, we have discovered that MAbs which induce greater crosslinking have more inhibitory activity (15,17). This has changed the way we and others screen MAbs for clinical use. As a result, we have generated homodimers of our MAbs and have found that their anti-proliferative activity on their specific target cells *in vitro* is profoundly increased (18). Furthermore, some nonsignaling MAbs can be converted to signaling MAbs following homodimerization. This is a novel and important finding for the MAb field which has significant clinical implications. The aim of this proposal was to determine whether IgG homodimers, or F(ab')₂ fragments of homodimers, of our highest affinity α -Her 2 monoclonal antibodies (MAbs) alone, or in combination, would make potent anti-tumor agents in SCID mice engrafted with Her-2⁺ human prostate cancer cell lines.

BODY:

Task 1: To prepare and test homodimers *in vitro* (grow up, purify, prepare homodimers and F(ab')₂ monomers and homodimers, determine K_A, K_D, structure and signaling on three cell lines.

Results: The affinity of our panel of α -HER-2 MAbs were determined by Scatchard analysis using BT474 cells, and the eleven with the best affinity, with K_Ds in the range of 10⁻⁸ to 10⁻¹⁰ M, are shown in Table 1. This panel of MAbs were shown to recognize eight non-crossblocking epitopes of HER-2.

Table 1. Characteristics of the Selected Panel of anti-HER-2 MABs

Non-cross blocking epitope recognized on the HER-2 molecule ¹	Anti-HER-2 MAB designation ²	Affinity (X 10 ⁹ M) ¹
A	50	0.2
A	158	6.7
B	66	0.1
C	70	2
D	80	1
E	81	0.1
E	112	3.3
E	143	10
F	156	6.7
G	164	0.1
H	157	3.3

¹Group designations randomly assigned as A-H.

²All are IgG1s, except HER156 which is an IgG2A.

³As determined by Scatchard analysis using BT474 cells.

Task 2: To characterize SCID/Xenograft model with respect to the best tumor to use, the route of administration, the growth pattern, metastases, PCR detection, endpoints etc.

Results: No progress has been made in this aim.

Task 3: To carry out all single and "cocktail" testing of homodimers and monomers on the prostate cell lines *in vitro*.

Results: Experimental results using three different α -Her-2 monomers and their respective dimers clearly showed that, in all cases, the dimers were much more effective inhibitors of proliferation at the concentrations tested (Figure 1), with IC₅₀s of 20 μ g/mL for either Her50 or HER66 dimers and 40 μ g/mL for HER70 dimer.

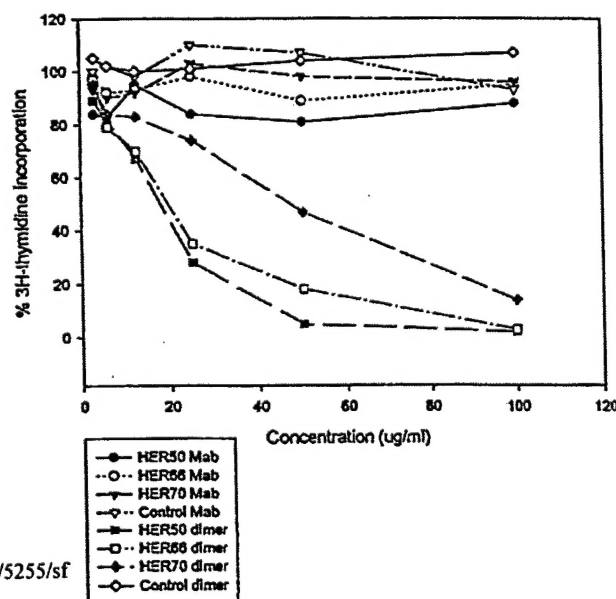


Figure 1. The effect of different MABs monomers or dimers on [³H]thymidine incorporation in LNCap cells, as described (19). Briefly, 5x10⁴ cells/well were treated for 24 hours with varying concentrations of antibody then pulsed with 1 μ Ci [³H]thymidine for 4 hours. Wells were harvested and the radioactivity retained in the cells counted and compared with untreated cell uptake (100%).

Task 4: To determine the biodistribution and pharmacokinetics of our best monomers/dimers of IgG and F(ab')₂ in SCID and SCID/Xenografted mice

Results: One of the HER-2 monomers, HER66, was injected into SCID mice and its biodistribution evaluated. As shown in Figure 2, there is considerable accumulation of material in the subcutaneous tumors. The testing of the other two anti-HER-2 antibodies is in progress.

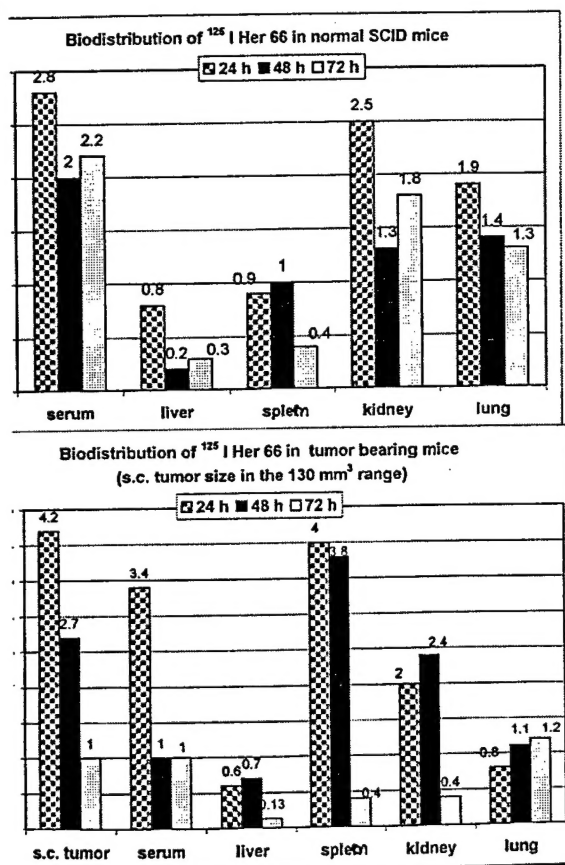


Figure 2. Biodistribution of ¹²⁵I HER66 in A) normal SCID mice, and B) SCID mice with 130 mm³ subcutaneous tumors. Radiolabeled α-Her2 MAb (HER66) was injected i.p. into 2 groups of 3 mice. Mice were sacrificed at 24, 48, and 72 hours. Perfused organs were weighed. The radioactivity of each organ as well as tumor and blood were measured. Results are expressed as % of injected dose per gram of tissue (%ID/g).

Task 5: To set up *in vivo* therapy experiments. The precise dose regimens will be selected based on biodistribution and pharmacokinetics

Results: Tasks 4 and 5 could not be completed being dependent on Task 2.

Task 6: cDNA cloning of the best hybridomas

Results: The variable domains of the heavy and light chains of six of the candidate anti-HER-2 antibodies (50, 66, 80, 81, 157, and 164) were PCR-amplified using a small set of primers specific for the leader sequence and the constant domain (these primers were

chosen to eliminate the possibility that slightly non-homologous variable domain PCR primers would alter the sequence). Only nine of these amplifications were successful, 50H, 50L, 66H, 66L, 80L, 81H, 157H, 157L, 164L, and of these, only four were cloned and sequenced to date, 50H, 50L, 66H, and 66L.

Task 7: Express appropriate multivalent Fv constructs of cDNAs in *E coli*. Check physicochemical properties of recombinant proteins.

Results: One of these constructs, which has an extra cysteine residue incorporated near the C-terminus of the heavy chain for disulfide bond formation of an antibody homodimer, has been successfully expressed in quantities sufficient to be converted into dimers using treatment with Elman's reagent. Characterization of this dimer, including avidity, specificity, and signaling properties will be evaluated and compared to the monomeric chimeric form. Two other chimeric constructs were described, one in which a cysteine residue was placed at the penultimate position of a 17-mer peptide at the C-terminal end of the heavy chain to facilitate homodimer formation, the other with an scFv of the same specificity genetically grafted onto the C-terminal of the heavy chain to generate a tetravalent construct. Both of these constructs have been successfully engineered, however, the yields from non-secreting mouse myeloma cell lines transfected by electroporation were never adequate for any characterization. Alternate transfection methods are being investigated that have since proven superior in our lab, including lipofection.

KEY RESEARCH ACCOMPLISHMENTS:

- The panel of anti-HER-2 MAbs was evaluated for binding affinity to HER-2 and the best eleven chosen for further evaluation.
- Three of the best anti-HER-2 MAbs, HER50, Her66 and HER70, were chemically dimerized and were shown to be significantly more cytotoxic than their corresponding monomers.
- A biodistribution study using HER66 showed that this antibody does accumulate in a tumor.
- Variable domain cloning of the light and heavy chain of six of the a-HER-2 MAbs has been initiated; two, HER50 and HER66 clones have been sequenced and await insertion into the chimeric, dimer expression vectors.
- Three different genetic dimer/multimer genetic constructs have been made and all express in transfected myeloma cells, though two of these in quantities that are not sufficient for evaluation.

REPORTABLE OUTCOMES: None

CONCLUSIONS:

We have identified three anti-HER-2 homodimers with antiproliferative activity on LnCAP cells *in vitro*. Because the generation of chemical homodimers is expensive, gives low yields and several biproducts, we are in the process of generating recombinant homodimers using three different strategies. One of these constructs has expression levels high enough to produce material for further evaluation. Six anti-HER-2 MAbs light and heavy chain variable domain regions are in various stages of cloning; these will be inserted into the best recombinant homodimer construct for expression. These genetic homodimers will be evaluated *in vitro* and *in vivo* using a SCID mouse xenograft model.

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APPENDICES: None